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CRITICAL WEIGHTS FOR METAMORPHOSIS IN THE TOBACCO HORNWORM, *MANDUCA SEXTA*

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Abstract

Fifth-instar larvae of the tobacco hornworm underwent a supernumerary larval molt under several feeding regimens. Supernumerary molting occurred only when the molt was initiated at a live weight less than about 3 g; at higher weights pupation took place. The supernumerary sixth instars were normal in appearance and behavior and went on to form normal pupae only when the fifth instar had begun at weights less than 1 g. Otherwise the supernumerary sixth instars exhibited precocious metamorphosis of their crochets and imaginal discs and failed to undergo normal pupation. Thus, the commitment to pupal development appears to occur in stages. A first stage, initiated at a weight of about 1 g, commits the crochets and imaginal discs to metamorphosis at the next molt. A second stage, entered at a weight of about 3 g, permits the full and complete pupation of the remaining tissues; this transition probably reflects the elimination of juvenile hormone (JH). Starvation apparently alters the normal patterns of ecdysone production, breakdown, or sensitivity so as to elicit ecdysone-dependent development at lower than normal weights. When starvation results in fifth-instar larvae initiating a developmental response at weights below 3 g, a supernumerary larval molt occurs. But in this case the critical effect of starvation is on the timing of the molt rather than on the JH titer, since the latter even in normally fed early fifth instars is high at weights below 3 g.

Introduction

Metamorphosis of the tobacco hornworm *Manduca sexta* normally occurs at the conclusion of the fifth instar. Nevertheless, experimental regimens of malnutrition can elicit one or even two additional larval molts (Nijhout, 1975a; Jones *et al.*, 1980; Cymborowski *et al.*, 1982). These supernumerary molts, like the four normal ones, require the concerted action of ecdysone and juvenile hormone (JH). Previous studies of these molts have focused primarily on the regulation of the hemolymph JH titer and the activity of the corpora allata (CA), both of which were high in fifth-instar larvae undergoing an extra larval molt but low in individuals initiating metamorphosis (Nijhout and Williams, 1974b; Nijout, 1975b; Bhaskaran and Jones, 1980; Cymborowski *et al.*, 1982). The high JH titer has been advanced as both a necessary and a sufficient cause of the extra larval molts (Bhaskaran and Jones, 1980; Cymborowski *et al.*, 1982).

The present study addresses this phenomenon anew. We have examined the effects of certain feeding regimens on the development of final-instar hornworms. The outcomes of these experiments argue against the earlier conclusion that supernumerary molting of malnourished fifth-instar larvae results from an atypical elevation of the JH titer. In the accompanying paper (Safranek and Williams, 1984) we demonstrate

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Abbreviations: JH, juvenile hormone; CA, corpora allata; LD, long day; SD, short day.

that larvae are regularly able to molt at much lower than predicted weights when they are malnourished. Here we suggest that a similar downward adjustment occurs as a response to malnourishment in larvae starved in the nominally final instar. When the adjustment is of sufficient magnitude, the molt begins at low body weights where the JH titer is high even in normally fed larvae. The resulting supernumerary larval molt thus reflects not an unusual variation in the JH titer but rather an alteration in the endocrine axis surrounding ecdysone that leads to molting at body weights below a threshold critical for metamorphosis.

Materials and Methods

Hornworms were reared at 25°C on an artificial diet as described by Truman (1972) and Bell and Joachim (1976) under either short-day (SD, 12L:12D) or long-day (LD, 17L:7D) photoperiods. Under these conditions the larvae routinely underwent five larval instars. Time-of-day was arbitrarily referenced to lights-off at midnight (24:00 = 00:00). The timing of events in the life cycle was as described by Truman (1972) and Truman and Riddiford (1974) except that we have termed the first 24 h of each instar Day 1 rather than Day 0.

Several different feeding regimens were employed in these experiments. In the "steady-weight regimen" larvae were weighed and returned to individual, clean, capped plastic containers which contained no food. Thereafter at daily intervals each individual was weighed and provided with an amount of diet sufficient to restore it to near its original weight. This regimen was maintained until molting or metamorphosis was initiated, as signaled, respectively, by apolysis of the head capsule or exposure of the dorsal vessel. In the "starvation-refeeding regimen" early fifth-instar larvae were weighed and returned to clean, individual, capped plastic containers where they were starved for one or more days. Thereafter they were restored to normal diet *ad lib* and observed daily for the onset of molting or metamorphosis.

Individuals molting to the sixth instar were subsequently observed under the dissecting microscope for a number of characters: the presence of pupal cuticle about the ocelli, antennae, and mouthparts; the appearance of pupal cuticle on the imaginal wing discs as seen through the translucent body wall; and the number, size, and shape of crochets on the prolegs. In the case of larvae dying prior to the onset of metamorphosis, the wing discs were excised to ascertain the presence or absence of a tanned, brittle, rugose, pupal-type cuticle.

Results

Effects of a "steady-weight" feeding regimen on final instar larvae

LD fifth-instar larvae were subjected to a steady-weight feeding regimen as described under Materials and Methods. Individuals were selected from each day of the instar up to 22:00 on the fourth day; all were therefore at least 4 h prior to the initiation of the pre-metamorphic endocrine events that culminate in the onset of the wandering period on the sixth day (Riddiford and Curtis, 1978). Larvae were maintained by daily feedings within .05 g of their weight at the outset of the regimen. All larvae underwent a molt. As depicted in Figure 1, those maintained at weights under 2.25 g formed supernumerary sixth-instar larvae after not less than 10 days (range: 10–25 days). By contrast, those held at steady weights ranging from 3.75 to 8.25 g exposed the dorsal vessel after less than 7 days and then formed normal pupae. Individuals held at weights between 2.75 and 3.25 g showed a variety of responses: some molted to sixth-instars, others wandered and subsequently formed normal albeit miniature pupae, still others molted to larval-pupal intermediates.

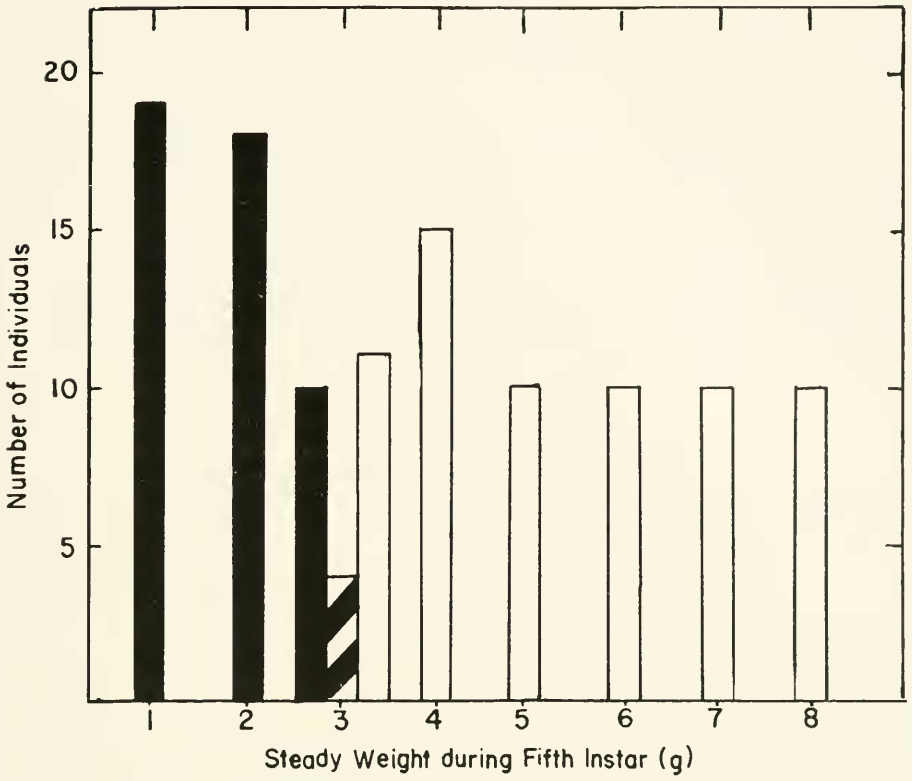

FIGURE 1. Transition from supernumerary larval molting to metamorphic development between 2 and 4 g in larvae maintained on a steady-weight regimen during the fifth instar. The number of larvae undergoing each type of development is shown as a function of the maintenance weight in the fifth instar. The various developmental outcomes were classified into 3 groups: (1) supernumerary sixth-instar larvae (black bars) lacked any traces of tanned pupal-like cuticle on epidermal structures other than the imaginal discs; (2) larval-pupal intermediates (hatched bars) exhibited regions of both larval-like and pupal-like cuticle; and (3) pupae (white bars) were smaller than normal but otherwise essentially normal in appearance and without traces of clear, flexible larval cuticle. Weight categories ranged ±0.25 g about the integral weights depicted in the figure. Details of this steady-weight regimen are described under Materials and Methods and Results.

Larvae molting to supernumerary sixth instars were never normal and none went on to pupate. The majority failed to undergo ecdysis or did so only partially and died without resuming feeding. The few which underwent successful ecdysis fed sporadically and ultimately died without further development. Attempts to salvage them by manually removing the old cuticle were to no avail since none resumed feeding. All exhibited one or more morphological abnormalities previously encountered in sixth instars resulting from juvenoid treatment of fifth instars (Trumen *et al.*, 1974). In the least affected individuals the crochets of one or more pairs of prolegs were noticeably reduced in size and number. The most abnormal lacked crochets entirely and had formed pupal cuticle on uneverted imaginal wing disks as well as on and about the ocelli and the everted imaginal discs of the antennae and mouthparts. Fewer abnormalities occurred when the maintenance weight was closer to 1 than to 3 g.

Among larvae destined to form larval-pupal intermediates an occasional individual exposed the dorsal vessel just prior to or even during apolysis and head capsule

slipping, but most did not. Regardless of the degree to which the dorsal vessel was exposed, all intermediates not only bore the just-described abnormalities characteristic of sixth-instar larvae but also displayed well-tanned, pock-marked pupal-like cuticle over variable but often extensive areas of the head, thorax, and abdomen.

In additional experiments we examined the development of about 100 fifth instars under short-day rather than the previous long-day conditions. Although the general pattern of response was the same as encountered in LD fifths, the critical weight for switching from a molting to a metamorphic response was nearer 2 than 3 g as under LD conditions. This difference withstood repeated scrutiny.

These experiments suggested that attainment of a threshold weight of about 2–3 g was a prerequisite for normal metamorphosis, but also that some metamorphic features were still to be found in the supernumerary larvae formed by fifth instars molting at even lower weights.

Effects of starvation-refeeding schemes on fifth-instar larvae

We next employed starvation-refeeding regimens of the type used to generate supernumerary instars in previous studies (Jones *et al.*, 1980; Cymborowski *et al.*, 1982). Thirty LD fifth-instar larvae were starved beginning on Day 1 at weights of 1.5–2.0 g. Thereafter groups of 10 were permitted to feed *ad lib* beginning on Days 4, 5, or 6 at about 18:00. All individuals were weighed daily at about 18:00 and observed for apolysis or dorsal vessel exposure.

The observations summarized in Figure 2 reveal that larvae destined to undergo a supernumerary larval molt typically grew more slowly than those that metamorphosed. The groups differed in two additional respects. Individuals undergoing a supernumerary molt were distinctly smaller at the time of apolysis (range: 2.5–5 g) than were metamorphosing larvae at the time of dorsal vessel exposure (range: 5–10 g). Moreover, apolysis occurred late on the fourth day of refeeding, whereas dorsal vessel exposure took place on the fifth or sixth day. If, in fact, initiation of the supernumerary molt in the refed larvae took place some 12–18 h prior to apolysis as is the case in normal larvae at the conclusion of the fourth instar (Truman, 1972; Cymborowski *et al.*, 1982), the molt would have been initiated typically at weights of about 3 to 3.5 g between Days 3 and 4 after feeding was resumed. The refed fifths that underwent metamorphosis were distinctly larger at a similar point after resumption of feeding, typically weighing 4–6 g between Days 3 and 4 of refeeding.

In a further series of experiments 30 LD fifth-instar larvae similar to those above were starved for 3, 4, or 5 days at the outset of the instar. But in this case at the end of the period of total starvation each larva was supplied daily only with sufficient food to maintain a steady weight of 1.5–2.0 g. In contrast to the earlier results none of these larvae initiated metamorphosis: all underwent a supernumerary larval molt, apolysis taking place 5–11 days after resumption of restricted feeding.

In both series of experiments the supernumerary sixth instars always showed faulty development of crochets as well as the localized zones of pupal cuticle described earlier. Many died without further development and those which eventually initiated metamorphosis inevitably formed non-viable pupae showing misshapen head and wing structures.

These experiments again implicated the attainment of a weight near 3 g as critical for metamorphosis. Additional experiments employing variants of this starvation-refeeding scheme revealed no significant differences from those cited here: in every case the weight interval from 2.0–4.0 g demarcated a zone of transition from a population of larvae capable of larval molting to one uniformly initiating metamorphosis.

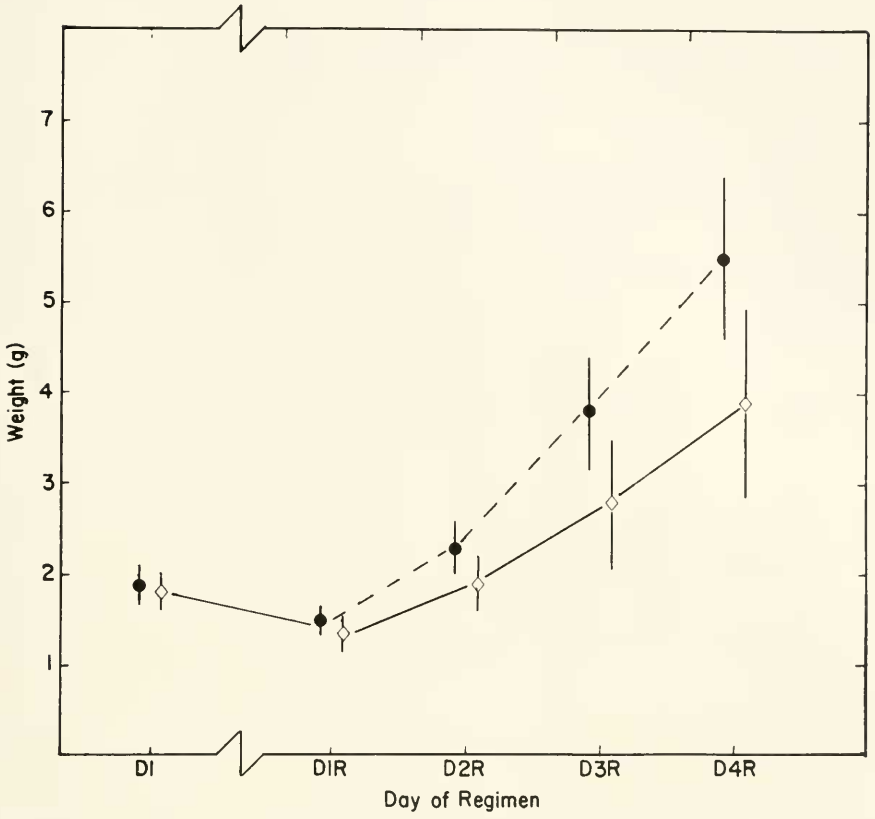

FIGURE 2. Relationship of fifth-instar larval growth rates under a starvation-refeeding protocol to the developmental outcome of the instar. The figure depicts the average weight ± S.D. of two groups of larvae on several days during the regimen. Larvae were removed from food during the last quarter of the first day of the instar (D1) and returned to food on Days 4–6 of the instar at about the same time of day (first day of refeeding for all groups: D1R). They were subsequently weighed daily during this same period. Twenty-two larvae (dark circles) exposed the dorsal vessel at weights of 5–9 g 96–120 h after resumption of feeding. Seven larvae (clear diamonds) molted to supernumerary sixth-instar larvae with head capsule slipping on Day 4 of refeeding.

Effects of malnourishment in the third and fourth instars

In a further series of experiments we removed fourth-instar SD larvae from food at weights from 0.30 to 0.90 g. They were starved during the remainder of the fourth instar except in the case of larvae under 0.60 g which were given small amounts of food to maintain them at approximately their initial weight so as to ensure their survival. All individuals were weighed again upon apolysis to the fifth instar. During the fifth instar they were fed *ad lib,* observed daily, and weighed at apolysis to a sixth instar or when dorsal vessel exposure signaled the onset of metamorphosis.

As shown in Figure 3, the weight at apolysis to the fifth instar was generally a strong predictor of the individual's fate at the conclusion of that instar. Larvae weighing more than 0.6 g almost without exception underwent metamorphosis, whereas those weighing less than 0.5 g more often underwent a supernumerary molt to a sixth instar. Figure 3 also depicts the weights at the end of the fifth instar. The vast majority of larvae initiating a molt to a sixth larval instar weighed less than 3.5 g and would

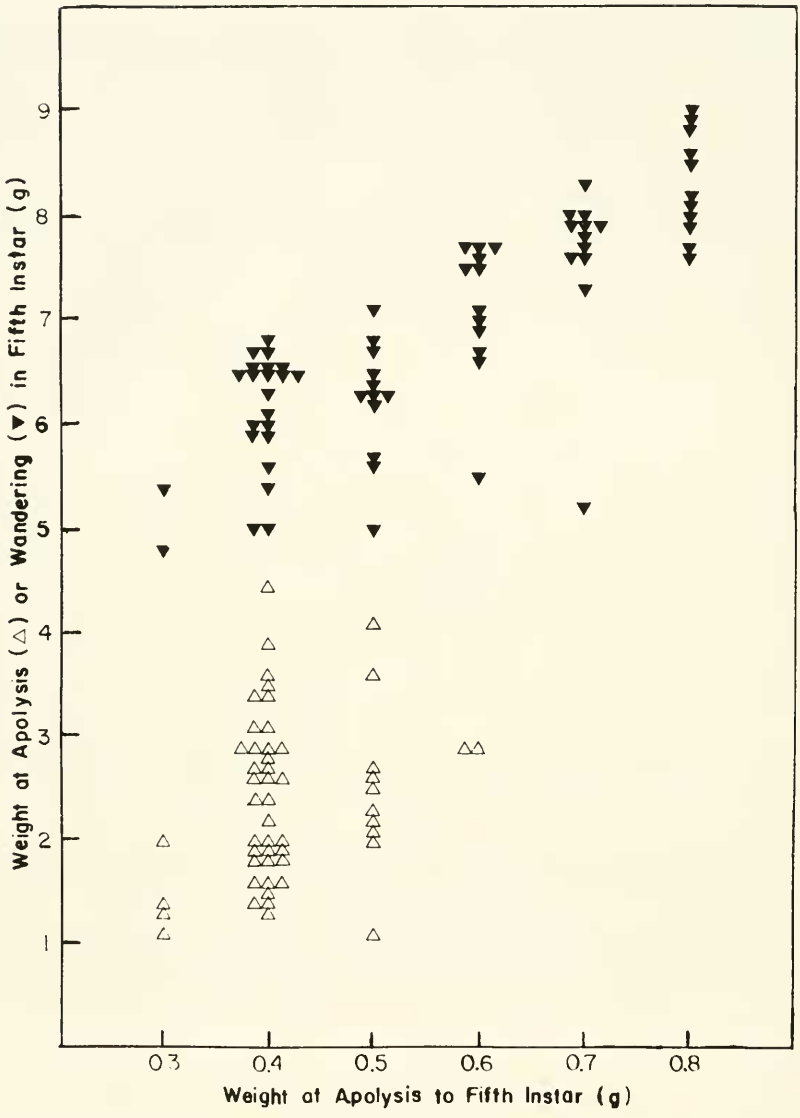

FIGURE 3. Transition from supernumerary larval molting to metamorphic development between 3 and 5 g. The live weight either at apolysis to the sixth instar (clear triangles) or at the time of initiation of the wandering period (dark triangles) is shown as a function of the same individual's weight at apolysis to the fifth instar. The range of sizes of the apolysing larvae was generated by partial starvation of the fourth instars. The weights recorded at the time of dorsal vessel exposure were undoubtedly lower than the maximum weight attained in the fifth instar by as much as 1 g due to the weight loss associated with gut evacuation and fluid loss at the outset of the wandering period.

have weighed approximately 0.5 g less at the time the molt was initiated about 12–18 h prior to apolysis (Trumen, 1972; Cymborowski *et al.*, 1982). Those weighing over 3.5 g nearly always underwent metamorphosis and with rare exception did so at weights over 5 g.

In a similar set of experiments third-instar SD larvae were held at 0.05–0.10 g until they molted to the fourth instar. These miniature fourths were then fed *ad lib* through both the fourth and fifth instars; their weights were subsequently recorded upon apolysis to the fifth instar and at apolysis or dorsal vessel exposure at the conclusion of the fifth instar.

Figure 4 depicts the fate of these larvae as a function of their weight at apolysis to the fifth instar. Similarities to the results of the experiments shown in Figure 3 are readily apparent. All larvae beginning the fifth instar above 0.6 g initiated metamorphosis, whereas those below 0.5 g underwent a supernumerary molt. Those undergoing a supernumerary molt did so at final weights under 2.0 g, whereas those initiating metamorphosis never weighed less than 6.0 g. The striking absence of

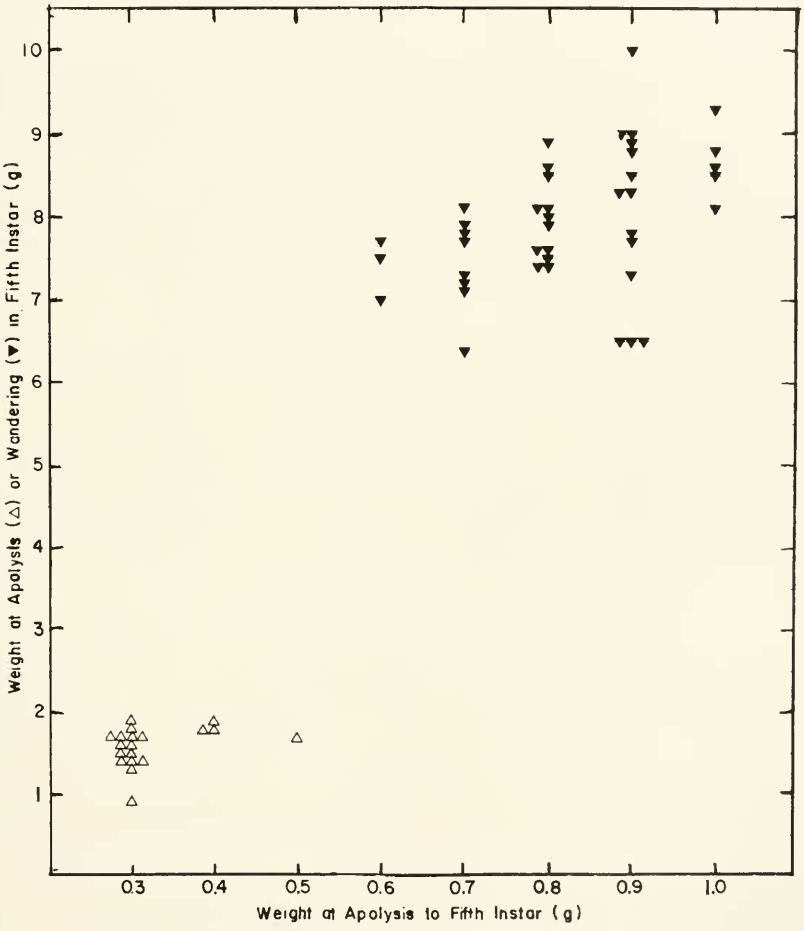

FIGURE 4. Transition from supernumerary larval molting to metamorphic development between 2 and 6 g. The live weight either at apolysis to the sixth instar (clear triangles) or at initiation of the wandering period (dark triangles) is shown as a function of the individual's weight at apolysis to the fifth instar. The range of sizes of the apolysing larvae was generated by prior partial starvation during the third instar. The weights recorded at the time of dorsal vessel exposure were undoubtedly lower than the maximum weight attained in the fifth instar by as much as 1 g due to the weight loss associated with gut evacuation and fluid loss at the outset of the wandering period.

development in the weight range of 2.0–6.0 g may be partly accounted for by a dearth of larvae entering the fifth instar between weights of 0.4–0.6 g—a finding encountered in the accompanying paper (Safranek and Williams, 1984).

In contrast to the aberrant supernumerary larvae resulting from starvation of normal fifth instars, the sixth instars formed from diminutive fifth-instar larvae appeared completely normal and eventually metamorphosed to pupae normal in appearance but for a slightly broader range of sizes.

These results pointed once again to the interval of 2–3 g as a critical threshold marking the transition from an endocrine milieu favoring a larval molt to one suitable for metamorphosis. But they also demonstrated that under some conditions a supernumerary molt could result in a normal sixth instar capable of normal metamorphosis and lacking the aberrations of crochets and imaginal discs noted in the sixths formed under the earlier regimens involving starvation of fifth instars.

Is the threshold relative or absolute?

In the experiments reported to this point, fifth-instar larvae underwent generalized metamorphosis only after attaining a weight of at least 2–3 g. In the accompanying paper (Safranek and Williams, 1984) we demonstrate that under normal culture conditions molting in earlier instars takes place only when a larva attains some multiple of its initial weight in that instar. We were curious as to whether the apparent threshold of 2–3 g represented a similar instance in which larvae were measuring their size relative to some standard set earlier in the instar or whether it represented an absolute threshold for the initiation of metamorphosis. This was examined in two sets of experiments.

In the first of these a group of fourth-instar SD larvae were held at a steady-weight of 0.65–0.75 g until they molted. A second group undergoing apolysis to the fifth instar was selected from stock in the weight range 1.3–1.6 g. Each of the two groups was further subdivided: some larvae were placed on a steady-weight regimen immediately upon ecdysis to the fifth instar; the others were fed and allowed to grow to some multiple of their initial weights before being placed on the steady-weight regimen. Table I records the results. In both groups the transition from larval to metamorphic development occurred at a maintenance weight of 2–2.2 g. Lower

TABLE I

Development of fifth-instar larvae held at various maintenance weights

Initial weight	Maintenance weight	Developmental fate*		
		Sixth-instar larvae	Larval-pupal intermediates	Pupae
0.65–0.75	1.3–1.5	12	0	0
1.3–1.5	1.3–1.5	12	0	0
0.65–0.75	1.6–1.9	12	2	0
0.65–0.75	2.0–2.2	0	7	5
1.3–1.6	2.0–2.2	3	5	4
0.65–0.75	2.7–3.0	0	0	12
1.3–1.6	2.7–3.0	0	1	11

* The number of larvae entering the several developmental pathways are indicated. Sixth-instar larvae had no traces of pupal cuticle except on their imaginal discs, where it appeared routinely. Larval-pupal intermediates presented a mosaic of larval and pupal cuticle. Pupae were without evidence of larval cuticle.

maintenance weights resulted only in supernumerary molting; higher weights, in metamorphosis.

In a second experiment fourth-instar SD larvae were subjected to a steady-weight regimen at weights of 0.4–0.5 g until they molted to the fifth instar, after which they were fed *ad lib.* The majority molted to sixth instars at weights from 1.6 to 3.5 g. These were then returned to steady-weight regimens either at their initial sixth-instar weight or after a brief period of feeding at a somewhat higher weight. Larvae maintained below 2.5 g almost always underwent a supernumerary molt whereas larvae above 3.0 g regularly metamorphosed (Fig. 5). Indeed, many of the larger larvae were able to undergo metamorphosis in the absence of any weight gain during the final instar.

In these two sets of experiments larvae molted to an additional larval instar when the molt was initiated below 2 g and underwent metamorphosis at weights beyond 3 g. This generalization held true even though larvae entered the nominally last instar over nearly a 3 g weight range from 0.65 to 3.5 g. Thus, the interval of 2–3 g appears to represent an absolute threshold whose passage is necessary and sufficient for metamorphosis. No evidence suggests that this passage occurs upon attainment of a multiple of the initial weight at the outset of the presumptive final instar.

Discussion

Two rules appear to govern the incidence and character of supernumerary molts by hornworm larvae under the feeding regimens employed here. First, an extra larval

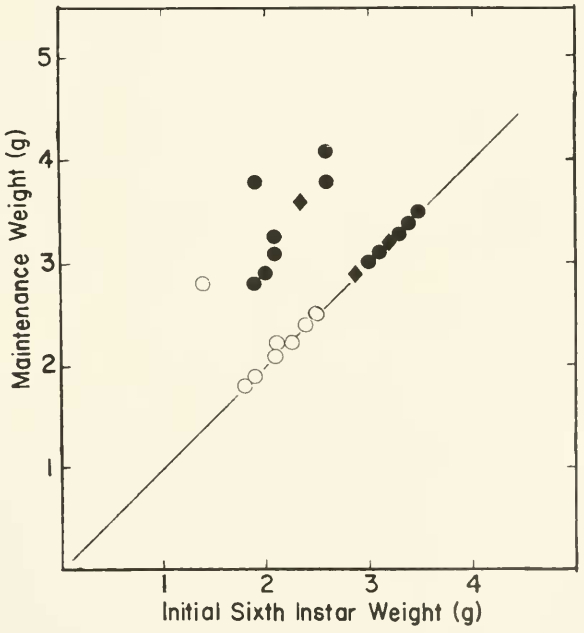

Figure 5. Developmental outcome of the sixth instar under a steady weight regimen at various maintenance weights as a function of the initial sixth-instar weight. Sixth-instar SD larvae were generated as described in Results and placed on a steady-weight regimen either at their initial body weight in that instar (data along solid line) or after an initial period of *ad lib* feeding (data above solid line). Larvae were observed daily for head-capsule slipping or dorsal vessel exposure, both of which occurred 10–28 days after initiation of the steady-weight regimen. After the molt individuals were judged to have formed a seventh larval instar (clear circles), a pupa (solid circles), or a larval-pupal intermediate (solid diamonds), the latter characterized by a mosaic pattern of larval- and pupal-like cuticle over the integument.

molt will be undertaken only if initiated at a weight below about 3 g; development initiated above 3 g follows a typical metamorphic sequence leading through the wandering period to pupation. Second, supernumerary sixth-instar larvae are morphologically aberrant and unable to pupate if they begin the preceding fifth instar at a weight above about 1 g.

The latter rule is supported by observations of fifth-instar larvae starved in the third or fourth instars. Those which entered the instar at a weight below 0.6 g regularly molted to sixths that were normal both in appearance and in their subsequent metamorphosis to pupae. These larvae respected both of the above rules: they initiated supernumerary molts at weights below 3 g and they entered the fifth instar at a weight below 1 g.

When, as under normal rearing conditions, *ad lib* feeding through the first four instars produced fifths weighing 1–1.5 g, no feeding protocol was then able to produce normal sixth instars: instead, the supernumerary larvae inevitably bore characteristic abnormalities reflecting the precocious metamorphosis of the crochets, the imaginal discs, and tissues about the head. These abnormalities were more pronounced as the supernumerary molt occurred at weights closer to 3 than to 1 g. Individuals so affected never metamorphosed to normal pupae. Prior investigations have disclosed a similar assortment of abnormalities in sixth-instar hornworms (Nijhout, 1976; Jones *et al.*, 1980; Cymborowski *et al.*, 1982). These results suggest the occurrence under normal circumstances of a developmental transition at the outset of the fifth instar which ensures the metamorphosis of the crochet epidermis and imaginal discs at the next molt without necessarily affecting the remainder of the integument.

The first of the above-mentioned rules describes a weight threshold for the induction of metamorphosis. This observation largely corroborates earlier work (Nijhout and Williams, 1974a) in which attention was drawn to the weight of 5 g: this was regarded as an approximate threshold size at which larvae could be starved and yet initiate metamorphosis synchronously with larvae fed *ad lib*—that is, without any delay. Here we direct attention to the acquisition of the lower weight between 2 and 3 g as an absolute prerequisite for the eventual metamorphosis of intact larvae. This threshold was most sharply defined in the experiments employing the steady-weight regimen in the final instar. A similar threshold could be discerned though less sharply after imposition of the steady-weight regimen in either the third or the fourth instars or under the starvation-refeeding schemes employed in the fifth instar in both this and prior studies (Jones *et al.*, 1980; Jones *et al.*, 1981; Cymborowski *et al.*, 1982). The apparent threshold defined by the latter regimens is likely to be higher and less well defined than that under a steady-weight regimen due to growth of the larva between the time the molt is initiated and the beginning of apolysis or between the time the threshold is crossed and the initiation of dorsal vessel exposure. For this reason, we consider the use of the steady-weight regimen in the normal final instar optimal for the determination of an accurate threshold size for metamorphosis.

Disparate frequencies of supernumerary molting in feeding-refeeding schemes have been reported in the several studies to date (Jones *et al.*, 1980; Jones *et al.*, 1981; Cymborowski *et al.*, 1982). Our results suggest that these differences may be attributed at least in part to the deployment of different photoperiods or to slight variations in the initial weight of fifth-instar larvae. Thus, in our experience SD larvae initiated metamorphosis at somewhat lower weights than LD larvae under otherwise identical regimens. Moreover, when larvae enter the fifth instar at weights around 0.7 g, starvation for 3 days followed by *ad lib* feeding always leads to a supernumerary larval molt (unpubl. obs.); by contrast, even 5 days of starvation failed to ensure larval molting of larvae entering the instar at 1–1.5 g in the experiments reported here.

Prior studies of the tobacco hornworm have emphasized the importance of the duration of malnutrition or of the composition of the food during the period of malnutrition. We are persuaded that the single major variable in regimens capable of eliciting a supernumerary molt is their ability to maintain fifth-instar larvae below the threshold of 2–3 g until the onset of a molt. Thus, full starvation of 1.5–2 g larvae for 3 days followed by maintenance on a steady-weight regimen at about 2 g always resulted in a supernumerary molt. By contrast, when similar larvae were allowed to feed *ad lib* after the 3 days of starvation, none underwent a supernumerary molt because they grew rapidly beyond the 3 g threshold for metamorphosis. Initial weight in the instar, weight at the time of starvation, and the duration of starvation are all likely to alter the incidence of supernumerary molting under any feeding regimen only insofar as they affect the size from which fifth-instar larvae undertake the subsequent molt.

The threshold of 3 g almost certainly marks the point of initiation of processes resulting in an irrevocable elimination of JH. Thus the hemolymph JH titer of normal larvae declines sharply beginning at 4–5 g—less than a day after reaching the threshold weight of 3 g (Nijhout and Williams, 1974b; unpubl. obs.). Furthermore the metamorphosis of the integument witnessed in larvae above 3 g can be largely prevented by topical administration of JH (Truman *et al.,* 1974). In normal larvae the loss of JH is likely to mirror accelerated breakdown of the hormone as well as its diminished production. The activity of JH esterase in both the hemolymph and tissues of fifth-instar *Manduca* increases beginning on Day 2 at about 3 g (Vince and Gilbert, 1977; Mitsui *et al.,* 1979; Beckage and Riddiford, 1981; unpubl. obs.). Moreover, a decline in CA activity has been noted about the same time (Bhaskaran *et al.,* 1980). The contribution of JH esterase to the metamorphosis of malnourished 3 g larvae in the current study was probably small: larvae of that size have relatively low levels of esterase even when fed *ad lib* and starvation typically induces a marked decline of prevailing esterase levels (Sparks *et al.,* 1983; unpubl. obs.). In all likelihood the present results reflect the onset of a decline of CA activity at a weight of about 3 g. In this regard we recall the suggestion of Bhaskaran *et al.* (1980) that a neurohumoral brain-derived factor released at a weight of approximately 3 g can curtail the endocrine activity of the CA.

Prior investigators of supernumerary molting induced by starvation have regarded the entire phenomenon as largely dependent on an atypical pattern of regulation of the CA and the JH titer (Bhaskaran and Jones, 1980; Cymborowski *et al.,* 1982). Data on the JH titer of starved fifth-instar larvae destined for a sixth instar indicate the level to be high during the period of starvation at 1 g and then to decline rapidly once feeding is resumed and the larvae approach weights of 3–4 g (Nijhout, 1975b; Cymborowski *et al.,* 1982). But this picture in fact resembles that of normal fifth instars where JH titers are initially quite high (Fain and Riddiford, 1975; Nijhout and Williams, 1974b). Although these elevated titers begin to decline shortly after ecdysis to the fifth instar, JH normally remains detectable at levels sufficient to block metamorphosis up to a weight of at least 5 g (Nijhout and Williams, 1974b; Nijhout, 1976). Since the vast majority of the hundreds of supernumerary molts observed in the course of the present study occurred at weights less than 3 g, the presence of JH at levels sufficient to ensure larval molting is hardly exceptional and is at best a precondition for supernumerary molting. Starvation-induced supernumerary molts reflect in considerable measure the ability of these feeding regimens to hold larvae at the low weights characteristic of the early final instar when JH levels are typically high. But the critical effect of starvation is to distort the usual size parameters associated with development in the final instar so that a molting gestalt is achieved at the low weights normally associated with high JH levels.

As we point out in the accompanying paper, starvation permits the achievement of a molting gestalt at much lower weights than occurs under conditions of normal feeding. In the fifth instar, as in earlier instars, starvation apparently short-circuits the normal machinery which links the onset of ecdysone-dependent development to the attainment of a critical size even to the extent of permitting larval molting in the absence of any growth during the instar. This could happen through several mechanisms: starvation may actively induce the endocrine changes that lead to accumulation of a molt-inducing titer of ecdysone or it may simply permit the passage of time to accomplish gradually what is completed promptly under conditions of normal growth. Different regimens can variously affect the duration of the instar prior to the onset of molting. Thus, fifth instars held at about 1 g initiated supernumerary molting after a minimum of 16 days and an average of nearly 3 weeks; by contrast, similar individuals totally starved for 5 days after ecdysis and then fed *ad lib* molted on either Day 8 or 9. Whatever the mechanism, this downward adjustment in the weight at which ecdysone-dependent development ensues has been noted after starvation in each instar examined (Safranek and Williams, 1984) and is necessary for the formation of a supernumerary instar by fifth-instar hornworms.

Supernumerary larval molting can be understood as the outcome of two facets of the hornworm's endocrine physiology: (1) the normal presence in all hornworms less than about 3 g of sufficient JH to permit a larval molt, and (2) the downward adjustment induced by starvation in the weight at which ecdysone-dependent development begins. When a large downward adjustment leads fifth-instar larvae to molt at weights below the critical threshold of 3 g, a supernumerary molt ensues; smaller adjustments result in the initiation of development at weights above this threshold where the JH level is too low to permit additional larval development, thus assuring metamophosis. This model suggests that a feeding regimen will tend to generate supernumerary larval molting to the degree that it can slow the growth rate and/or accelerate the generation of a molting gestalt in fifth-instar larvae. In this view, malnutrition does not bring about a supernumerary larval molt primarily through peculiar effects on the JH titer; rather, it serves to fix the individual in an endocrine milieu appropriate for additional larval development until changes in ecdysone production, metabolism, or sensitivity initiate a molt.

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